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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> (21) International Application Number: PCT/US99/12252 (22) International Filing Date: 2 June 1999 (02.06.99) </div> <div style="width: 50%;"> (71) Applicant (for all designated States except US): GENENTECH, INC. [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BAKER, Kevin [GB/US]; 14006 Indian Run Drive, Darnestown, MD 20878 (US). CHEN, Jian [CN/US]; 22-03 Hunters Glen Drive, Plainsboro, NJ 08536-3854 (US). GODDARD, Audrey [CA/US]; 110 Congo Street, San Francisco, CA 94131 (US). GURNEY, Austin, L. [US/US]; 1 Debbie Lane, Belmont, CA 94002 (US). SMITH, Victoria [AU/US]; 19 Dwight Road, Burlingame, CA 94010 (US). WATANABE, Colin, K. [US/US]; 128 Corliss Drive, Moraga, CA 94556 (US). WOOD, William, I. [US/US]; 35 Southdown Court, Hillsborough, CA 94010 (US). YUAN, Jean [CN/US]; 176 West 37th Avenue, San Mateo, CA 94403 (US). (74) Agents: KRESNAK, Mark, T. et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (US). (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). </div> </div>																																																																				
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> (30) Priority Data: <table style="width: 100%; border-collapse: collapse;"> <tr><td style="width: 30%;">60/087,607</td><td style="width: 30%;">2 June 1998 (02.06.98)</td><td style="width: 40%;">US</td></tr> <tr><td>60/087,609</td><td>2 June 1998 (02.06.98)</td><td>US</td></tr> <tr><td>60/087,759</td><td>2 June 1998 (02.06.98)</td><td>US</td></tr> <tr><td>60/087,827</td><td>3 June 1998 (03.06.98)</td><td>US</td></tr> <tr><td>60/088,021</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,025</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,028</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,029</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,030</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,033</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,326</td><td>4 June 1998 (04.06.98)</td><td>US</td></tr> <tr><td>60/088,167</td><td>5 June 1998 (05.06.98)</td><td>US</td></tr> <tr><td>60/088,202</td><td>5 June 1998 (05.06.98)</td><td>US</td></tr> <tr><td>60/088,212</td><td>5 June 1998 (05.06.98)</td><td>US</td></tr> <tr><td>60/088,217</td><td>5 June 1998 (05.06.98)</td><td>US</td></tr> <tr><td>60/088,655</td><td>9 June 1998 (09.06.98)</td><td>US</td></tr> <tr><td>60/088,722</td><td>10 June 1998 (10.06.98)</td><td>US</td></tr> <tr><td>60/088,730</td><td>10 June 1998 (10.06.98)</td><td>US</td></tr> <tr><td>60/088,734</td><td>10 June 1998 (10.06.98)</td><td>US</td></tr> <tr><td>60/088,738</td><td>10 June 1998 (10.06.98)</td><td>US</td></tr> <tr><td>60/088,740</td><td>10 June 1998 (10.06.98)</td><td>US</td></tr> <tr><td>60/088,741</td><td>10 June 1998 (10.06.98)</td><td>US</td></tr> </table> <p style="text-align: center; margin-top: 10px;"><i>(Continued after the drawings)</i></p> </div> <div style="width: 50%;"> <p>Published <i>Without international search report and to be republished upon receipt of that report.</i></p> </div> </div>			60/087,607	2 June 1998 (02.06.98)	US	60/087,609	2 June 1998 (02.06.98)	US	60/087,759	2 June 1998 (02.06.98)	US	60/087,827	3 June 1998 (03.06.98)	US	60/088,021	4 June 1998 (04.06.98)	US	60/088,025	4 June 1998 (04.06.98)	US	60/088,028	4 June 1998 (04.06.98)	US	60/088,029	4 June 1998 (04.06.98)	US	60/088,030	4 June 1998 (04.06.98)	US	60/088,033	4 June 1998 (04.06.98)	US	60/088,326	4 June 1998 (04.06.98)	US	60/088,167	5 June 1998 (05.06.98)	US	60/088,202	5 June 1998 (05.06.98)	US	60/088,212	5 June 1998 (05.06.98)	US	60/088,217	5 June 1998 (05.06.98)	US	60/088,655	9 June 1998 (09.06.98)	US	60/088,722	10 June 1998 (10.06.98)	US	60/088,730	10 June 1998 (10.06.98)	US	60/088,734	10 June 1998 (10.06.98)	US	60/088,738	10 June 1998 (10.06.98)	US	60/088,740	10 June 1998 (10.06.98)	US	60/088,741	10 June 1998 (10.06.98)	US
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(54) Title: MEMBRANE-BOUND PROTEINS AND NUCLEIC ACIDS ENCODING THE SAME																																																																				
(57) Abstract <p>The present invention is directed to polypeptides and to nucleic acid molecules encoding those polypeptides. Also provided herein are vectors and host cells comprising those nucleic acid sequences, chimeric polypeptide molecules comprising the polypeptides of the present invention fused to heterologous polypeptide sequences, antibodies which bind to the polypeptides of the present invention and to methods for producing the polypeptides of the present invention.</p>																																																																				

interactions, and cellular adhesion molecules like selectins and integrins. For instance, transduction of signals that regulate cell growth and differentiation is regulated in part by phosphorylation of various cellular proteins. Protein tyrosine kinases, enzymes that catalyze that process, can also act as growth factor receptors. Examples include fibroblast growth factor receptor and nerve growth factor receptor.

In light of the important physiological roles played by ATPases and membrane-bound proteins efforts are being undertaken by both industry and academia to identify new, native membrane-bound proteins, and proteins having sequence identity to ATPases. We herein describe the identification and characterization of novel polypeptides having sequence identity to GST ATPase, designated herein as PRO203 polypeptides.

9. PRO290

Of particular interest are novel proteins and nucleic acids which have sequence identity with known proteins and nucleic acids. Proteins of interest which are well known in the art include NTII-1, a nerve protein which facilitates regeneration, FAN, and beige. Beige, or bg, is a murine analog related to Chediak-Higashi Syndrome (CHS), a rare autosomal recessive disease in which neutrophils, monocytes and lymphocytes contain giant cytoplasmic granules. See Perou et al., *J. Biol. Chem.* 272(47):29790 (1997) and Barbosa et al., *Nature* 382:262 (1996).

We herein describe the identification and characterization of novel polypeptides having sequence identity to NTII-1, FAN and beige, designated herein as PRO290 polypeptides.

10. PRO874

Efforts are being undertaken by both industry and academia to identify new, native membrane-bound proteins. Many of these efforts are focused on the screening of mammalian recombinant DNA libraries to identify the coding sequences for novel membrane-bound proteins. We herein describe the identification and characterization of novel transmembrane polypeptides, designated herein as PRO194 polypeptides.

11. PRO710

In *Saccharomyces cerevisiae*, the chromatin structure of DNA replication origins changes as cells become competent for DNA replication, suggesting that G1 phase-specific association of replication factors with origin DNA regulates entry into S phase (Aparicio et al., *Cell* 91:59-69 (1997)). In fact, it has been shown that the initiation of DNA replication in *Saccharomyces cerevisiae* requires the protein product of the CDC45 gene which encodes a protein that stays at relatively constant levels throughout the cell cycle (Owens et al., *Proc. Natl. Acad. Sci USA* 94:12521-12526 (1997)). The CDC45 protein is part of a prereplication complex that may move with DNA replication forks in yeast. Given the obvious importance of the CDC45 protein in DNA replication, there is significant interest in identifying and characterizing novel polypeptides having homology to CDC45. We herein describe the identification and characterization of novel polypeptides having homology to the CDC45 protein, designated herein as PRO710 polypeptides.

monitoring a biological activity mediated by said polypeptide.

In a still further embodiment, the invention concerns a composition comprising a PRO290 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a pharmaceutically acceptable carrier.

10. PRO874

5 Applicants have identified a cDNA clone that encodes a novel multi-span transmembrane polypeptide, which is designated in the present application as "PRO874".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO874 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO874 polypeptide having amino acid residues 1 to 321 of Figure 25 (SEQ ID NO:36), or is complementary to such
10 encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. In other aspects, the isolated nucleic acid comprises DNA encoding the PRO874 polypeptide having amino acid from about X to 321 of Figure 25 (SEQ ID NO:36), where X is any amino acid from about 270 to about 279 of Figure 25 (SEQ ID NO:36), or is complementary to such encoding nucleic acid
15 sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. The isolated nucleic acid sequence may comprise the cDNA insert of the DNA40621-1440 vector deposited on June 2, 1998, as ATCC 209922 which includes the nucleotide sequence encoding PRO874.

In another embodiment, the invention provides isolated PRO874 polypeptide. In particular, the invention provides isolated native sequence PRO874 polypeptide, which in one embodiment, includes an amino acid sequence comprising residues 1 to 321 of Figure 25 (SEQ ID NO:36). Additional embodiments of the
20 present invention are directed to PRO874 polypeptides comprising amino acids X to 321 of Figure 25 (SEQ ID NO:36), where X is any amino acid from about 270 to about 279 of Figure 25 (SEQ ID NO:36). Optionally, the PRO874 polypeptide is obtained or is obtainable by expressing the polypeptide encoded by the cDNA insert of the DNA40621-1440 vector deposited on June 2, 1998, as ATCC 209922.

25 11. PRO710

Applicants have identified a cDNA clone that encodes a novel polypeptide having homology to CDC45 protein, wherein the polypeptide is designated in the present application as "PRO710".

In one embodiment, the invention provides an isolated nucleic acid molecule comprising DNA encoding a PRO710 polypeptide. In one aspect, the isolated nucleic acid comprises DNA encoding the PRO710
30 polypeptide having amino acid residues 1 to 566 of Figure 27 (SEQ ID NO:41), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. In other aspects, the isolated nucleic acid comprises DNA encoding the PRO710 polypeptide having amino acid residues about 33 to 566 of Figure 27 (SEQ ID NO:41) or amino acid 1 or about 33 to X of Figure 27 (SEQ ID NO:41), where X is any amino acid from 449 to 458 of Figure 27 (SEQ ID
35 NO:41), or is complementary to such encoding nucleic acid sequence, and remains stably bound to it under at least moderate, and optionally, under high stringency conditions. The isolated nucleic acid sequence may comprise the cDNA insert of the DNA44161-1434 vector deposited on May 27, 1998 as ATCC 209907 which

sequence of amino acid residues 1 to about 229, inclusive of Figure 306 (SEQ ID NO:424).

In a further aspect, the invention concerns an isolated PRO1384 polypeptide, comprising an amino acid sequence scoring at least about 80% positives, preferably at least about 85% positives, more preferably at least about 90% positives, most preferably at least about 95% positives when compared with the amino acid sequence of residues 1 to 229 of Figure 306 (SEQ ID NO:424).

5 In yet another aspect, the invention concerns an isolated PRO1384 polypeptide, comprising the sequence of amino acid residues 1 to about 229, inclusive of Figure 306 (SEQ ID NO:424), or a fragment thereof sufficient to provide a binding site for an anti-PRO1384 antibody. Preferably, the PRO1384 fragment retains a qualitative biological activity of a native PRO1384 polypeptide.

10 In a still further aspect, the invention provides a polypeptide produced by (i) hybridizing a test DNA molecule under stringent conditions with (a) a DNA molecule encoding a PRO1384 polypeptide having the sequence of amino acid residues from about 1 to about 229, inclusive of Figure 306 (SEQ ID NO:424), or (b) the complement of the DNA molecule of (a), and if the test DNA molecule has at least about an 80% sequence identity, preferably at least about an 85% sequence identity, more preferably at least about a 90% sequence identity, most preferably at least about a 95% sequence identity to (a) or (b), (ii) culturing a host cell comprising
15 the test DNA molecule under conditions suitable for expression of the polypeptide, and (iii) recovering the polypeptide from the cell culture.

In yet another embodiment, the invention concerns agonists and antagonists of a native PRO1384 polypeptide. In a particular embodiment, the agonist or antagonist is an anti-PRO1384 antibody.

20 In a further embodiment, the invention concerns a method of identifying agonists or antagonists of a native PRO1384 polypeptide, by contacting the native PRO1384 polypeptide with a candidate molecule and monitoring a biological activity mediated by said polypeptide.

In a still further embodiment, the invention concerns a composition comprising a PRO1384 polypeptide, or an agonist or antagonist as hereinabove defined, in combination with a pharmaceutically acceptable carrier.

25 136. Additional Embodiments

In other embodiments of the present invention, the invention provides vectors comprising DNA encoding any of the above or below described polypeptides. A host cell comprising any such vector is also provided. By way of example, the host cells may be CHO cells, *E. coli*, or yeast. A process for producing any of the above or below described polypeptides is further provided and comprises culturing host cells under
30 conditions suitable for expression of the desired polypeptide and recovering the desired polypeptide from the cell culture.

In other embodiments, the invention provides chimeric molecules comprising any of the above or below described polypeptides fused to a heterologous polypeptide or amino acid sequence. An example of such a chimeric molecule comprises any of the above or below described polypeptides fused to an epitope tag sequence
35 or a Fc region of an immunoglobulin.

In another embodiment, the invention provides an antibody which specifically binds to any of the above or below described polypeptides. Optionally, the antibody is a monoclonal antibody.

sequence with other known nucleotide sequences using any of a number of well known sequence alignment programs and determining which PRO polypeptide-encoding nucleotide sequence fragment(s) are novel. All of such PRO polypeptide-encoding nucleotide sequences are contemplated herein. Also contemplated are the PRO polypeptide fragments encoded by these nucleotide molecule fragments, preferably those PRO polypeptide fragments that comprise a binding site for an anti-PRO antibody.

- 5 In another embodiment, the invention provides isolated PRO polypeptide encoded by any of the isolated nucleic acid sequences hereinabove identified.

- In a certain aspect, the invention concerns an isolated PRO polypeptide, comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet
10 more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity, yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about
15 about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to a PRO polypeptide having a full-length amino acid sequence as disclosed herein, a full-length amino acid sequence lacking the signal peptide as disclosed herein or an
20 extracellular domain of a transmembrane protein as disclosed herein.

- In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid sequence having at least about 80% sequence identity, preferably at least about 81% sequence identity, more preferably at least about 82% sequence identity, yet more preferably at least about 83% sequence identity, yet more preferably at least about 84% sequence identity, yet more preferably at least about 85% sequence identity,
25 yet more preferably at least about 86% sequence identity, yet more preferably at least about 87% sequence identity, yet more preferably at least about 88% sequence identity, yet more preferably at least about 89% sequence identity, yet more preferably at least about 90% sequence identity, yet more preferably at least about 91% sequence identity, yet more preferably at least about 92% sequence identity, yet more preferably at least about 93% sequence identity, yet more preferably at least about 94% sequence identity, yet more preferably at
30 least about 95% sequence identity, yet more preferably at least about 96% sequence identity, yet more preferably at least about 97% sequence identity, yet more preferably at least about 98% sequence identity and yet more preferably at least about 99% sequence identity to an amino acid sequence encoded by any of the human protein cDNAs deposited with the ATCC as disclosed herein.

- In a further aspect, the invention concerns an isolated PRO polypeptide comprising an amino acid
35 sequence scoring at least about 80% positives, preferably at least about 81% positives, more preferably at least about 82% positives, yet more preferably at least about 83% positives, yet more preferably at least about 84% positives, yet more preferably at least about 85% positives, yet more preferably at least about 86% positives,

an isolated PRO polypeptide nucleic acid molecule includes PRO polypeptide nucleic acid molecules contained in cells that ordinarily express the PRO polypeptide where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-PRO monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-PRO antibody compositions with polyepitopic specificity, single chain anti-PRO antibodies, and fragments of anti-PRO antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mMsodiumphosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M

include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native PRO polypeptides, peptides, small organic molecules, etc. Methods for identifying agonists or antagonists of a PRO polypeptide may comprise contacting a PRO polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the PRO polypeptide.

5 "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to
10 an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats,
15 rabbits, etc. Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often
20 the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including
25 glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv
30 fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-combining sites
35 and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific
5 for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge
10 region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant
15 domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).
20

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH - VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and
25 Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).
30

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1)
35 to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or

nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

5 The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody so as to generate a "labeled" antibody. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

10 By "solid phase" is meant a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

15 A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a PRO polypeptide or antibody thereto) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

A "small molecule" is defined herein to have a molecular weight below about 500 Daltons.

20 II. Compositions and Methods of the Invention

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO polypeptides. In particular, cDNAs encoding various PRO polypeptides have been identified and isolated, as disclosed in further detail in the Examples below. It is noted that proteins produced in separate expression rounds may be given different PRO numbers but the UNQ number is unique for any given DNA and the encoded protein, and will not be changed. However, for sake of simplicity, in the present specification the protein encoded by the full length native nucleic acid molecules disclosed herein as well as all further native homologues and variants included in the foregoing definition of PRO, will be referred to as "PRO/number", regardless of their origin or mode of preparation.

25 As disclosed in the Examples below, various cDNA clones have been deposited with the ATCC. The actual nucleotide sequences of those clones can readily be determined by the skilled artisan by sequencing of the deposited clone using routine methods in the art. The predicted amino acid sequence can be determined from the nucleotide sequence using routine skill. For the PRO polypeptides and encoding nucleic acids described herein, Applicants have identified what is believed to be the reading frame best identifiable with the sequence information available at the time.

35 1. Full-length PRO281 Polypeptides

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO281 (UNQ244). In particular, cDNA encoding a PRO281

10. **Full-length PRO874 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO874. In particular, Applicants have identified and isolated cDNA encoding a PRO874 polypeptide, as disclosed in further detail in the Examples below. The PRO874-encoding clone was isolated from a human fetal lung library. To Applicants present knowledge, the DNA40621-1440
5 nucleotide sequence encodes a novel factor. Although, using BLAST and FastA sequence alignment computer programs, some sequence identity with known proteins was revealed.

11. **Full-length PRO710 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO710. In particular, Applicants have identified and isolated cDNA
10 encoding a PRO710 polypeptide, as disclosed in further detail in the Examples below. Using BLAST and FastA sequence alignment computer programs, Applicants found that the PRO710 polypeptide has significant similarity to the CDC45 protein. Accordingly, it is presently believed that PRO710 polypeptide disclosed in the present application is a newly identified CDC45 homolog.

12. **Full-length PRO1151 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1151. In particular, cDNA encoding a PRO1151 polypeptide has
15 been identified and isolated, as disclosed in further detail in the Examples below.

Using the WU-BLAST-2 sequence alignment computer program, it has been found that a full-length native sequence PRO1151 (shown in Figure 30 and SEQ ID NO:47) has certain amino acid sequence identity with the human 30 kD adipocyte complement-related precursor protein (ACR3_HUMAN). Accordingly, it is presently believed that PRO1151 disclosed in the present application is a newly identified member of the complement protein family and may possess activity typical of that family.

13. **Full-length PRO1282 Polypeptides**

The present invention provides newly identified and isolated nucleotide sequences encoding polypeptides referred to in the present application as PRO1282. In particular, cDNA encoding a PRO1282 polypeptide has
20 been identified and isolated, as disclosed in further detail in the Examples below.

As far as is known, the DNA45495-1550 sequence encodes a novel factor designated herein as PRO1282. Using WU-BLAST-2 sequence alignment computer programs, some sequence identities between PRO1282 and other leucine rich repeat proteins were revealed, as discussed in the examples below, indicating that a novel member of the leucine rich repeat superfamily has been identified.

14. **Full-length PRO358 Polypeptides**

The present invention further provides newly identified and isolated nucleotide sequences encoding a polypeptide referred to in the present application as PRO358. In particular, Applicants have identified and
35

or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

3. Human and Humanized Antibodies

The anti-PRO antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., *Nature*, 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers

specifically, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced homology between the PRO203 amino acid sequence and the following Dayhoff sequences, AF008124_1, CFRCD1GEN_1, and P_R82566.

EXAMPLE 12: Isolation of cDNA clones Encoding Human PRO290

- 5 An expressed sequence tag (EST) DNA database (LIFESEQ®, Incyte Pharmaceuticals, Palo Alto, CA) was searched and an EST was identified that had homology to beige and FAN. An oligonucleotide probe based upon the identified EST sequence was then synthesized and used to screen human fetal kidney cDNA libraries in an attempt to identify a full-length cDNA clone. The oligonucleotide probe had the following sequence:
5' TGACTGCACTACCCCGTGGCAAGCTGTTGAGCCAGCTCAGCTG 3' (SEQ ID NO:34).
- 10 RNA for construction of cDNA libraries was isolated from human fetal kidney tissue. The cDNA libraries used to isolate the cDNA clones encoding human PRO290 were constructed by standard methods using commercially available reagents such as those from Invitrogen, San Diego, CA. The cDNA was primed with oligo dT containing a NotI site, linked with blunt to SalI hemikinased adaptors, cleaved with NotI, sized appropriately by gel electrophoresis, and cloned in a defined orientation into a suitable cloning vector (such as pRKB or pRKD; pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., Science 253:1278-1280 (1991)) in the unique XhoI and NotI.
- 15 A cDNA clone was identified and sequenced in entirety. The entire nucleotide sequence of DNA35680-1212 is shown in Figure 22 (SEQ ID NO:32). Clone DNA35680-1212 contains a single open reading frame with an apparent translational initiation site at nucleotide positions 293-295, and a stop codon at nucleotide positions 3302-3304 (Figure 22; SEQ ID NO:32). The predicted polypeptide precursor is 1003 amino acids long.

- It is currently believed that the PRO290 polypeptide is related to FAN and/or beige. Clone DNA35680-1212 has been deposited with ATCC and is assigned ATCC deposit no. 209790. It is understood that the deposited clone has the actual correct sequence rather than the representations provided herein. The full-length PRO290 protein shown in Figure 23 has an estimated molecular weight of about 112,013 daltons and a pI of about 6.4.
- 25

EXAMPLE 13: Isolation of cDNA Clones Encoding Human PRO874

- A consensus DNA sequence designated herein as DNA36459 was identified using phrap as described in Example 1 above. Based on the DNA36459 consensus sequence, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the coding sequence for PRO874.
- 30

PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-TCGTGCCCCAGGGGCTGATGTGC-3' (SEQ ID NO:37); and
reverse PCR primer 5'-GTCTTTACCCAGCCCCGGGATGCG-3' (SEQ ID NO:38).

- 35 Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA36459 sequence which had the following nucleotide sequence:

hybridization probe

5'-GGCCTAATCCAACGTTCTGTCTTCAATCTGCAAATCTATGGGGTCCTGGG-3' (SEQ ID NO:39).

In order to screen several libraries for a source of a clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO874 gene using the probe oligonucleotide and one of the PCR primers. RNA for construction of the cDNA libraries was isolated from human fetal lung tissue (LIB25).

DNA sequencing of the clones isolated as described above gave the DNA sequence for PRO874 [herein designated as DNA40621-1440] (SEQ ID NO:35) and the derived protein sequence for PRO874.

The entire nucleotide sequence of DNA40621-1440 is shown in Figure 24 (SEQ ID NO:35). Clone DNA40621-1440 contains a single open reading frame ending at the stop codon at nucleotide positions 964-966 (Figure 24). The predicted polypeptide encoded by DNA40621-1440 is 321 amino acids long (Figure 25). The PRO874 protein shown in Figure 25 has an estimated molecular weight of about 36,194 daltons and a pI of about 9.85. Analysis of the PRO874 sequence shown in Figure 25 (SEQ ID NO:36) evidenced the presence of the following: a type II transmembrane domain at about amino acids 57-80; additional transmembrane domains at about amino acids 110-126, 215-231, and 254-274; potential N-glycosylation sites at about amino acids 16-19, 27-30, and 289-292; sequence identity with hypothetical YBR002c family proteins at about amino acids 276-287; and sequence identity with ammonium transporter proteins at about amino acids 204-230. Clone DNA40621-1440 was deposited with the ATCC on June 2, 1998, and is assigned ATCC deposit no. 209922.

Analysis of the amino acid sequence of the PRO874 polypeptide suggests that it is a novel multi-span transmembrane protein. However, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced sequence identity between the PRO874 amino acid sequence and the following Dayhoff sequences: S67049, AF054839_1, S73437, S52460, and HIVU80570_1.

EXAMPLE 14: Isolation of cDNA Clones Encoding Human PRO710

A yeast screening assay was employed to identify cDNA clones that encoded potential secreted proteins. Use of this yeast screening assay allowed identification of a single cDNA clone whose sequence (herein designated as DNA38190) is shown in Figure 28 (SEQ ID NO:42). Based on the DNA38190 sequence shown in Figure 28, oligonucleotides were synthesized: 1) to identify by PCR a cDNA library that contained the sequence of interest, and 2) for use as probes to isolate a clone of the full-length coding sequence for PRO710. In order to screen several libraries for a full-length clone, DNA from the libraries was screened by PCR amplification, as per Ausubel et al., Current Protocols in Molecular Biology, with the PCR primer pair. A positive library was then used to isolate clones encoding the gene of interest using the probe oligonucleotide and one of the primer pairs.

PCR primers (forward and reverse) were synthesized:

forward PCR primer 5'-TTCCGCAAAGAGTTCTACGAGGTGG-3' (SEQ ID NO:43)
reverse PCR primer 5'-ATTGACAACATTGACTGGCCTATGGG-3' (SEQ ID NO:44)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the DNA38190 sequence which had the following nucleotide sequence